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Cognitive Performance and Iron Status are Negatively Associated with Hookworm Infection in Cambodian Schoolchildren

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Abstract. Soil-transmitted helminth (STH) infection has been associated with lower cognitive performance of schoolchildren. To identify pathways through which STH infection might affect school performance, baseline data from a large rice-fortification trial in Cambodian schoolchildren were used to investigate associations between STH infection, micronutrient status, anemia, and cognitive performance. Complete data on anthropometry, cognitive performance, and micronutrient status were available for 1,760 schoolchildren, 6–16 years of age. STH infection was identified using Kato–Katz, whereas cognitive performance was assessed using Raven's Colored Progressive Matrices (RCPM), block design, and picture completion. STH infection was found in 18% of the children; almost exclusively hookwork infection. After adjusting for age and gender, raw cognitive test scores were significantly lower in hookworm-infected children (−0.65; −0.78; −2.03 points for picture completion, RCPM, and block design, respectively; $P < 0.05$ for all). Hookworm infection was associated with iron status (total body iron), but not with vitamin A and zinc status, nor with inflammation or anthropometry. Body iron was negatively associated with increased intensity of hookworm infection ($R = 0.22$, $P < 0.001$). Hookworm infection in Cambodian schoolchildren was associated with lower cognitive performance, an effect most likely mediated through lower body iron. Interventions that are more effective against hookworm infection are needed to contribute to better health and improvement of cognitive performance.

INTRODUCTION

Soil-transmitted helminth (STH) infections are a significant public health problem in most low- and middle-income countries in Asia, Africa, and Latin America.[1] It is estimated that a third of the world's population is infected with one or more species of intestinal helminths, which may impair children's nutritional status, growth, and development.[2] Studies have shown helminth infection to be associated with undernutrition, stunted growth, iron-deficiency anemia (IDA), poor school attendance, and poor performance in cognition tests.[3–6] School-aged children are considered particularly at risk to STH infection,[7] often having the highest intensity and prevalence of infection.[8] Both the World Bank and World Health Organization (WHO) promote helminth control programs in developing countries as a cost-effective intervention.[9]

Although several studies have examined associations between helminth infection and children's academic performance or cognitive function, the findings have been inconsistent. Indeed, despite the apparent convincing studies and recommendations, a recent Cochrane review concluded that there is hardly any benefit for blanket distribution of deworming medication for children living in STH-endemic areas, with no benefit for anthropometry, hemoglobin concentrations, cognitive development, or school attendance.[10] This Cochrane review did not look specifically at different STH species, whereas we recently showed that different STH species can have different effects, for example, on micronutrient status.[11]

Among STHs, the hookworm species, *Necator americanus* and *Ancylostoma duodenale*, are estimated to infect about 600 million people globally with the highest infection intensity among children between 5 and 15 years of age.[12] In a number of developing countries, hookworm is the leading underlying cause of anemia due to blood loss and consequent iron deficiency (ID).[13] Hookworm infection causes mechanical laceration and enzymatic damage to the mucosa of the small intestine leading to approximately 0.05 mL/day of blood loss per adult *N. americanus* and approximately 0.25 mL/day per adult *A. duodenale*.[14] If blood loss exceeds the dietary iron absorption, ID and later IDA will develop. Typically, a hypochromic microcytic anemia follows chronic infection within 3–5 months after the infection.[7]

ID and IDA have been linked to impaired psychomotor development in children[15,16] and reduced work capacity in adults.[17] Although some of the effects of ID on cognitive functioning appear to be permanent, improvements in cognition have been reported after iron supplementation in other studies.[18] Although hookworm infection can cause ID through direct blood loss, *Ascaris* and *Trichuris* infection have also been associated with ID,[19] but mechanisms are less well understood.

In Cambodia, despite a considerable reduction in national poverty since the mid-1990s, undernutrition remains a major problem. The 2010 Cambodia Demographic and Health Survey reported that 40% of children are stunted, 11% are wasted, and 55% of children of 6–59 months are anemic.[20] Cambodia is considered to have one of the highest STH prevalence rates in southeast Asia; however, national prevalence data are not available. A national school-based health program, including twice annual school-based distributions of single-dose mebendazole (500 mg),[21] has been in operation since 2002, but recent surveys still show that up to 27.6% of primary-school children are infected with intestinal helminthes. Before the national deworming program, *Ascaris lumbricoides* infection rates ranged between 24.8% and 62.2%, and hookworm infection rates between 15.3% and 68.9%.[22]

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In view of the abovementioned Cochrane review that concluded that helminth control had little impact on nutritional status, school performance, or cognition,[10] we investigated whether associations between STH, micronutrient status, and cognitive performance existed in Cambodian schoolchildren. The aim of our study was foremost to quantify STH prevalence in Cambodian schoolchildren, and to identify pathways through which helminth infection might affect school performance.

METHODS

Study design and sample. The study was conducted within primary schools in Kampong Speu, a province located 40 km west of the Phnom Penh Capital where livelihoods are predominantly in the agriculture sector, although in recent years, labor in the garment and textile industrial sector has increased significantly. Twenty schools were randomly selected from primary schools participating in school meal program (16 selected of 18 overall) or take-home ration program (four selected of 96 overall) of the United Nations World Food Program. Data were collected between mid-November and mid-December 2012. Children were eligible for inclusion if written informed consent was obtained from their parents or caretakers. From each grade (1–6), 22 eligible children (11 boys and 11 girls) were randomly selected before visiting the school. In total, 2,640 children were randomly identified. Of these, 197 children were not recruited due to their absence on the day of data collection or refusal to participate. A total of 2,443 schoolchildren, 6–16 years of age, participated in the study, and only children with complete blood and stool samples collected (1,760 children) were included in the present analysis.

The study was approved by the National Ethic Committee for Health Research of the Ministry of Health, Phnom Penh, Cambodia, the Ministry of Education, Youth and Sports, Phnom Penh, Cambodia, and the Research Ethics Committee of PATH, Seattle, WA.

Anthropometric measurements. The anthropometric measurements obtained included height and weight. A training course and standardization test was administered to the team before data collection. Height and weight measurements were obtained using standardized procedures.[23,24] Children wore minimal clothes and no shoes. Height was measured twice to the nearest 0.1 cm using a wooden stadiometer. Weight was measured once, to the nearest 0.1 kg using an electronic scale (Seca, 881 U, GmbH & Co. KG, Hamburg, Germany). Height-for-age z-score (HAZ) and body mass index–for-age z-score (BAZ) were calculated with AnthroPlus software version 1.0.4 (World Health Organization, Geneva, Switzerland) using the WHO 2007 standards. Stunting and thinness were respectively defined as $-3 < \text{HAZ} < -2$ and $-3 < \text{BAZ} < -2$, and severe stunting and severe thinness as $\text{HAZ} < -3$ and $\text{BAZ} < -3$, respectively.

Cognitive performance tests. The cognitive test was translated and adapted into the local Khmer language by a team of experts from the Department of Psychology, Royal University of Phnom Penh (RUPP). The cognitive test was then administered by students of this same department. The RUPP students were given a week-long training and standardization test with primary-school children in Phnom Penh (not the research area), before the research study started. Each primary school child was tested individually and the cognitive test scores were interpreted as raw scores since standardized norms are not available for Cambodia. For all cognitive tests, higher scores indicate better performance.

Three main tests were used for cognitive assessment: the Raven's Colored Progressive Matrices test (RCPM), and two standardized tests from the Wechsler Intelligence Scale for Children (WISC III): block design and picture completion. RCPM, the colored form of Raven's Progressive Matrices test for use with children, is a widely used nonverbal test of intelligence which was designed as a measure of overall intellectual ability.[25,26] The WISC III, designed for children 6–16 years of age, is one of the most widely used tests of the intelligence of children.[27] The block design test is a measure of problem solving to assess executive functions. Picture completion evaluates alertness to detail and visual discrimination.

Blood and urine sample. Blood samples were taken from the antecubital vein between 8:00 AM to 12:00 PM using venipuncture following the standard protocol. Blood (5 mL) was stored in trace element–free vacutainers with no anticoagulant (Vacuette; Greiner Bio One GmbH, Kremsmünster, Austria). Urine samples were collected from the children in a sterile plastic container. Blood and urine samples were stored in cool boxes kept cool with plastic ice packs at a temperature of < 5°C and transported to Phnom Penh within 5 hours of collection. At the laboratory facility in Phnom Penh, the blood samples were centrifuged at 2,700 rpm ($1{,}300 \times g$) for 10 minutes at room temperature. Serum and urine samples were then aliquoted in capped Eppendorf tubes and stored at −25°C until transfer for analysis.

Hemoglobin concentration. Hemoglobin concentrations were determined at the primary school during data collection, immediately after blood sampling using the HemoCue (301 + system; HemoCue Angelholm, Sweden). Anemia was defined as having a hemoglobin concentration < 115 g/L for children between 6 and 11 years of age, < 120 g/L for children between 12 and 14 years of age and girls ≥ 15 years of age, and < 130 g/L for boys ≥ 15 years of age, according to WHO guidelines.[28]

Body iron. The diagnosis of ID was tedious in our cohort because of the wide difference in the estimates of ID prevalence between the two biomarkers of iron status, with ferritin (FER) indicating < 2% of ID and transferrin receptor (TfR) suggesting > 50% of ID. Ferritin concentrations are regarded to reflect iron stores, mainly in the liver and macrophages, whereas TfR reflects tissue need for iron.[29,30] Earlier studies from Cambodia also reported that biomarkers for iron status are less reliable in Cambodia due to the high prevalence of hemoglobinopathies.[31] We have therefore opted to report on FER and TfR as descriptive data, but have used body iron to assess the impact of iron status on cognition, as body iron is a composite of both FER and TfR.[32]

Laboratory analysis. Serum samples were sent on dry ice to the VitMin laboratory (Willstaett, Germany) for determination of retinol-binding protein (RBP), C-reactive protein (CRP), FER, soluble TfR, and α-1-acid-glycoprotein (AGP), and to National Institute of Nutrition (Hanoi, Vietnam) for zinc analysis. RBP, FER, TfR, CRP, and AGP were measured by a sandwich enzyme-linked immunosorbent assay technique.[33] Zinc concentration was measured using a flame atomic absorption spectrophotometer (GBC, Avanta+, GBC Scientific Equipment, Braeside VIC, Australia) using trace

element–free procedures. Inflammation was defined as high CRP (> 5 mg/L) and/or high AGP concentrations (> 1 g/L). Inflammation status was then categorized in four groups based on CRP and AGP levels: no inflammation (normal CRP and AGP), incubation (high CRP and normal AGP), early convalescence (high CRP and AGP), and late convalescence (normal CRP and high AGP).[34] FER is affected by presence of infection or inflammation, therefore FER concentrations were adjusted using correction factors published by Thurnham and others, namely 0.77, 0.53, and 0.75 for children in incubation, early convalescence, and late convalescence phases, respectively.[34] Low FER (corrected value < 15 μg/L) was used as an indicator of depleted iron stores,[35,36] and high TfR (> 8.3 mg/L) as an indicator of iron tissue deficiency.[37,38] ID was defined using both FER and TfR indicators, that is, by depleted iron stores or iron tissue deficiency. Body iron was calculated from FER corrected for inflammation and TfR, as described by Cook and others.[39] Serum retinol is bound to RBP in a 1-to-1 complex, hence RBP concentrations were used to evaluate vitamin A (VA) status.[40] RBP concentrations were adjusted for the presence of inflammation using correction factors of 1.15, 1.32, and 1.12 for incubation, early convalescence and late convalescence phases, respectively.[41] Corrected RBP cutoffs were used to define marginal VA status (< 1.05 μmol/L), vitamin A deficiency (VAD, < 0.70 μmol/L), and severe VAD (< 0.35 μmol/L), respectively.[40,42] Zinc deficiency was defined using the following cutoffs: serum zinc < 9.9 μmol/L for age 4–9 years, < 10.1 μmol/L for girls ≥ 10 years of age, and < 10.7 μmol/L for boys ≥ 10 years of age.[43] Severe zinc deficiency was defined as serum zinc < 7.6 μmol/L. Diagnosis of hemoglobinopahy was performed using automated capillary zone electrophoresis (Capillarys 2: Sebia, Lisses, France)[44] at the Institute of Pasteur, Cambodia.

STH infection. Stool sample from children was collected once. Plastic containers and instructions for fecal sample collection were distributed to the children on the day of data collection and requested to be returned with fecal sample to the school the following day. Samples were then stored in a cool box, transported to the National Malaria Center (Phnom Penh, Cambodia), and stored at 4°C until analysis. Quantitative examination for intestinal helminth eggs was performed using the WHO modification of the Kato–Katz method.[45] Two Kato smears were prepared from each stool sample, using 41.7 mg of stool sample each time. The egg output was expressed as eggs per gram feces (epg).

Socioeconomic survey. Socioeconomic information was collected on a subsample (N = 454 children) by trained interviewers during household visits. Questionnaires were answered by parents or caregivers and included information about household characteristics, caregiver's level of education, and amount and source of household income.

Statistical analyses. Data analysis was performed using SPSS software version 22.0 (IBM Corporation, Armonk, NY). Normality of distributions was evaluated using Kolmogorov–Smirnoff test. Continuous variables with skewness and kurtosis values outside the range of −1.0 to +1.0 were log transformed. General linear regression model was used to quantify the association between cognitive performance of schoolchildren and helminth infection. Binary logistic regression was performed to evaluate the association between STH infection and various health indicators including micronutrient deficiency status, inflammation, and anthropometric indices. The odds ratio (OR) for each association was adjusted for age, sex, marginal VA status, zinc deficiency, inflammation, hemoglobinopathy, and anemia. Linear regression analysis was performed with body iron and base 10 logarithm of hookworm infection intensity (epg). For analysis of anemia risk factors and to explore the association between STH infection, cognitive function, and anemia, logistic regression was performed with two models: one model that excluded body iron and one model that included body iron. Bonferroni post hoc adjustment was used for the multiple comparisons.

RESULTS

Hookworm among other STH infections. The overall STH infection rate among participating children was 18%, with boys more likely to be infected than girls (20% and 16%, respectively; P < 0.031). STH infection was not associated with the age of the child. The vast majority of infections were caused by hookworm infection (95%), whereas the prevalence of *Ascaris* or *Trichuris* infection was low. Very few cases of multiple infection were observed (< 0.1%) such as hookworm coinfected with *Trichuris* or hookworm with *Taenia*. Overall, most infections were of light intensity (Table 1).

Characteristics of the studied sample by hookworm infection. The mean age of the study sample was 9.8 years

TABLE 1
Overall STH infection (number of infected children)

Parameter	Male	Female	All n (%)	P value
N = 1,760	857	903		
Overall parasite infection	174 (20%)	147 (16%)	321 (18)	0.031
Hookworm*	160 (19%)	128 (14%)	288 (17)	0.012
Light	156 (97.5%)	122 (95.3%)	278 (96.5)	
Moderate/severe	4 (1.9%)	6 (4.7%)	10 (3.1)	
Ascaris	1 (0.1%)	4 (0.2%)	5 (0.3)	0.378
Light (all < 5,000 epg)	1 (0.1%)	4 (0.2%)	5 (0.3)	
Trichuris	1 (0.1%)	4 (0.2%)	5 (0.3)	0.378
Light (all < 1,000 epg)	1 (0.1%)	4 (0.2%)	5 (0.3)	
Others (*Taenia*, *Fasciola*, *Enteo*, *Hydimi*, *Hynana*)	20 (1.1%)	17 (1.1%)	37 (2.1)	
Multi-infection				
Hookworm + *Trichuris*	0	2 (<0.1%)	2 (<0.1)	
Hookworm + *Taenia*	5 (<0.1%)	3 (<0.1%)	8 (<0.1)	

epg = egg per gram feces; STH = soil-transmitted helminth.
*Hookworm: light < 2000 epg, moderate = 2,000–4000 epg, severe > 4,000 epg.

(range from 6–15 years). The prevalence of underweight was 31.5%, including 11.6% of the children being severely underweight. The prevalence of stunting was 28.9%, including 13.2% with severe stunting. Inflammation (elevated concentration of CRP and/or AGP) was common and affected more than one-third (38.1%) of the children. There was no significant difference in anthropometric status or prevalence of inflammation between hookworm-infected and noninfected children. The median household income was 1,815 USD/household/year. Children with hookworm infection were more likely to come from a household with a lower average annual income than households from children without hookworm infection ($P < 0.05$), but education of the caregiver was not related to hookworm infection.

The prevalence of anemia was 15.1% but almost none (0.1%) of the children had severe anemia (Table 2). The prevalence of ID, as defined by low FER and/or high TfR, was 51%, mainly due to high TfR concentrations, whereas the prevalence of IDA was 9.1%. Prevalence of children with negative total body iron stores (TBI < 0) was 1.5%, with another 9.7% of the children having marginal body iron stores (0 ≤ TBI < 4 mg/kg body weight). In children with hookworm infection, the prevalence of anemia was significantly higher, as well as the prevalence of low FER and low or marginal body iron was higher as compared with noninfected children ($P < 0.01$ for all). The prevalence of VAD was 0.9%, with another 8.6% of the children having marginal VA status (0.7 μmol/L ≤ RBP < 1.01 μmol/L). Almost all (91.3%) the children were classified as zinc deficient, and approximately half (51%) were classified as severely zinc deficient, based on serum zinc concentrations. There was no significant difference of VA or zinc status between hookworm-infected and noninfected children.

Children with hookworm infection had significantly lower scores in all cognitive tests compared with noninfected children ($P < 0.05$ for picture completion and Raven test, and $P < 0.001$ for block design).

Cognitive performance and hookworm infection. General linear regression analyses showed that hookworm infection was significantly associated with cognitive performance of

TABLE 2
Characteristics of schoolchildren by hookworm infection

Parameter	Noninfected by hookworm	Infected by hookworm	All	*P* value
N	1,450	288	1,738	
Age	9.82 ± 2.19	9.88 ± 2.25	9.83 ± 2.20	0.654
% Inflammation	37.7 (*N* = 538)	37.2 (*N* = 109)	37.8 (*N* = 647)	0.894
Anthropometric status				
WAZ (only for < 10 years)	−1.88 ± 0.93 (*N* = 783)	−1.81 ± 0.93 (*N* = 155)	−1.87 ± 0.93 (*N* = 938)	0.364
% WAZ < −2SD	31.0 (*N* = 243)	34.1 (*N* = 53)	31.5 (*N* = 938)	
% WAZ < −3SD	11.8 (*N* = 93)	10.3 (*N* = 16)	11.6 (*N* = 109)	
HAZ	−1.81 ± 1.05	−1.83 ± 1.09	−1.81 ± 1.06	0.691
% HAZ < −2SD	28.6 (*N* = 416)	30.5 (*N* = 88)	28.9 (*N* = 503)	
% HAZ < −3SD	13.1 (*N* = 190)	13.9 (*N* = 40)	13.2 (*N* = 230)	
BAZ	−1.50 ± 0.89	−1.44 ± 0.86	−1.49 ± 0.89 (*N* = 1,738)	0.257
% BAZ < −2SD	27.8 (*N* = 403)	24.3 (*N* = 70)	27.2 (*N* = 471)	
% BAZ < −3SD	0.0 (*N* = 1)	0.0 (*N* = 0)	0.0 (*N* = 1)	
Micronutrient status				
Hb (g/L)	124.47 ± 9.2	122.33 ± 11.2	124.43 ± 9.6 (*N* = 1,738)	< 0.001
% Anemia	14.3 (*N* = 207)	20.5 (*N* = 59)	15.3 (*N* = 1,738)	< 0.01
% Severe anemia	0.00 (*N* = 0)	0.01 (*N* = 2)	0.01 (*N* = 2)	< 0.01
FER*† (μg/L)	80.83 ± 37.88	58.65 ± 29.61	77.13 ± 37.55	< 0.001
% FER* < 15 μg/L	0.6 (*N* = 9)	3.1 (*N* = 9)	1.1 (*N* = 18)	< 0.01
TfR† (mg/L)	8.65 ± 2.33	9.42 ± 3.84	8.78 ± 2.66)	< 0.001
% TfR > 8.3 mg/L	49.9 (*N* = 711)	56.3 (*N* = 161)	50.9 (*N* = 872)	< 0.05
% ID‡ total	50.0 (*N* = 714)	56.6 (*N* = 162)	51.1 (*N* = 876)	< 0.05
% ID‡ with anemia	7.9 (*N* = 113)	15.7 (*N* = 45)	9.2 (*N* = 102)	< 0.05
Body iron (mg/kg)	6.77 ± 1.96	5.23 ± 2.85	6.51 ± 2.20	< 0.001
% Body iron < 0	0.7 (*N* = 10)	6.7 (*N* = 19)	1.7 (*N* = 29)	< 0.001
% Body iron < 4	6.8 (*N* = 99)	24.7 (*N* = 71)	9.8 (*N* = 170)	< 0.001
RBP* (μmol/L)	1.59 ± 0.43	1.54 ± 0.43	1.58 ± 0.43	0.074
% Marginal VA status§	8.1 (*N* = 116)	10.8 (*N* = 31)	8.6 (*N* = 147)	0.132
% Zinc deficiency‡‡	91.0 (*N* = 1,093)	92.5 (*N* = 222)	91.26 (*N* = 1,315)	0.455
Cognitive scores (raw score adjusted by age)				
Picture completion	8.12 ± 4.7	7.58 ± 4.48	8.03 ± 4.67	< 0.05
Block design	14.9 ± 9.82	13.14 ± 9.32	14.6 ± 9.76	< 0.001
Raven progressive test	17.46 ± 4.89	16.87 ± 4.75	17.34 ± 4.87	< 0.05
Socioeconomic status of household (on a subgroup *N* = 454)				
Income§§ ($/year/household)	1,825 (375, 4,290)	1,672 (262, 2,940)	1,815 (375, 4,147)	< 0.05
% No or informal schooling (caregiver)	15.3 (*N* = 60)	20.0 (*N* = 10)	15.8 (*N* = 70)	0.819
% Primary school (caregiver)	61.3 (*N* = 241)	54.0 (*N* = 27)	60.5 (*N* = 268)	
% Secondary school (caregiver)	23.4 (*N* = 92)	26.0 (*N* = 50)	23.7 (*N* = 105)	

BAZ = BMI-for-age z-score; BMI = body mass index; FER = ferritin; HAZ = height-for-age z-score; Hb = hemoglobin; ID = iron deficiency; RBP = retinol-binding protein; SD = standard deviation; TfR = transferrin receptor; UIC = urinary iodine concentration; VA = vitamin A; VAD = vitamin A deficiency; WAZ = weight-for-age z-score. Results are mean ± SD, unless otherwise stated.

*Corrected for inflammation.

†Geometric mean ± SD.

‡Based on FER* < 15 μg/L and/or TfR 0.8.3 mg/L.

§0.7 ≤ RBP* < 1.05 μmol/L.

‡‡Serum zinc < 9.9 μmol/L for age 4–9 years, < 10.1 μmol/L for girls ≥ 10 years of age, < 10.7 μmol/L for boys ≥ 10 years of age.

§§Median (10th; 90th).

schoolchildren (Table 3). After adjusting for age and gender (Model 1), the raw scores on the three cognitive tests were significantly lower in infected children compared with the noninfected children. For picture completion and Raven progressive matrix test, children with hookworm scored −0.65 and −0.78 points lower, respectively ($P < 0.05$), than children without infection. Hookworm infection appeared to have an even stronger effect on the block design results with infected children having block design scores −2.03 points lower ($P < 0.001$) than uninfected children. We explored different pathways on how hookworm infection could have affected the cognitive performance of the schoolchildren. Inclusion of inflammation, zinc, and VA (Model 2) did not affect the associations that were found between hookworm infection and cognitive test scores. Moreover, all these factors were not associated with any of the cognitive tests. However, when including body iron in the model (Model 3), hookworm infection was no longer significantly associated with picture completion and less strongly associated with the other two cognitive scores. In a separate analysis, socioeconomic status was also included in the above models, but showed no significant association with cognitive scores (data not shown).

Health risks associated with hookworm infection. Hookworm infection was significantly associated with iron status (TBI), but not with other biomarkers of micronutrient status (VA and zinc), nor with systemic infection or anthropometric status (Table 4). Binary logistic regression showed that having a hookworm infection gave much higher risks for negative or marginal body iron (body iron < 4 mg/kg). For example, children with hookworm infection were six times (OR = 6.3) more likely to have a negative body iron than uninfected children.

The intensity of hookworm infection among infected children was also associated with body iron, with body iron significantly lower with increased intensity of hookworm infection ($R = 0.22$, $P < 0.001$). In the linear regression model, body iron among infected children was 1.27 mg/kg lower for each increase of a unit of log hookworm epg. A similar association was observed between Hb concentration and intensity of hookworm infection ($R = 0.22$, $P < 0.001$), with Hb concentration becoming lower with increased hookworm epg.

Hookworm infection among risk factors for anemia. Anemia has a multifactorial pathogenesis, and it is apparent that in this population, ID does not account for most of the anemia observed, since most of the children who participated in the study had replete iron stores (FER > 15 μg/L). Indeed, other significant factors for anemia in the schoolchildren were hemoglobinopathy, which affected 44.5% of the children and deficient or marginal VA status. One possible explanation for the high iron stores in the presence of anemia could be inflammation. Inflammation leads to a redistribution of iron from the circulation to the macrophages, and if this condition becomes chronic, gives rise to the so-called "anemia of chronic disease." To test whether hookworm infection, through induction of a systemic inflammatory response, could have led to anemia, we ran a multivariable regression model with and without body iron. In the model without body iron, hookworm infection was a significant determinant of anemia ($P < 0.001$), even when controlling for systemic inflammation (Table 5). However, in the model with body iron, hookworm infection was no longer statistically significant, whereas body iron became a strong predictor of anemia ($P < 0.001$).

TABLE 4

Multivariable regression of health indicators in association with hookworm infection

Total $N = 1,738$ Variables	n	Odds ratio	95% CI for odds ratio	P value
Body iron < 0*	22	6.3**	2.5–15.8	< 0.001
Body iron < 4†	137	4.4**	3.0–6.5	< 0.001
Marginal VA status‡	147	1.4	0.9–2.0	0.149
Zinc deficiency§	1,315	1.2	0.7–2.0	0.479
Inflammation¶	647	1.0	0.8–1.3	0.921
HAZ < −2SD	503	1.1	0.8–1.4	0.438
WAZ < −2SD	938	1.0	0.7–1.5	0.755

AGP = α-1-acid-glycoprotein; CI = confidence interval; CRP = C-reactive protein; HAZ = height-for-age z-score; RBP = retinol-binding protein; VA = vitamin A; WAZ = weight-for-age z-score.

*Deficit iron store (body iron < 0 mg/kg of body weight).

†Low iron store (body iron < 4 mg/kg of body weight).

‡0.7 ≤ RBP1 < 1.05 μmol/L.

§Serum zinc < 9.9 μmol/L for age 4–9 years, < 10.1 μmol/L for girls ≥ 10 years of age, < 10.7 μmol/L for boys ≥ 10 years of age.

¶Elevated CRP/AGP or both CRP and AGP.

**Adjusted for age, sex, marginal VA status, zinc deficiency, inflammation, hemoglobinopathy, and anemia.

DISCUSSION

In our study, hookworm infection in schoolchildren was significantly associated with lower scores in all three cognitive tests. Most of the schoolchildren with STH had a hookworm infection, whereas infection with other helminths (i.e., *Ascaris*) was uncommon. Before the national deworming program, the prevalence of *Ascaris* infection ranged from

TABLE 3

Cognitive performance in association with hookworm infection ($N = 1,738$)

		Picture completion		Block design		Raven progressive	
Model	Variables in the model	Adjusted mean difference	P value	Adjusted mean difference	P value	Adjusted mean difference	P value
Model 1	Cognitive raw score	−0.65	0.016	−2.03	0.000	−0.78	0.005
Model 2	Cognitive raw score Inflammation Zinc Vitamin A	−0.67	0.029	−2.10	0.001	−0.94	0.003
Model 3	Cognitive raw score Inflammation Zinc Vitamin A Body iron	−0.41	0.193	−1.64	0.01	−0.72	0.004

Mean difference = mean of infected − noninfected. All models adjusted by age and sex.

TABLE 5
Multivariable regression analysis of hookworm infection among risk factors for anemia

	Gender		Hookworm infection	Hemoglobinopathy*	Marginal VA status†	Zinc deficiency‡	Inflammation§	Low body iron¶
	(Ref: male)	Age	(Ref: noninfected)	(Ref: HbE < 5%)	(Ref: normal VA status)	(Ref: normal zinc status)	(Ref: no inflammation)	(Ref: normal body iron)
n (*N* = 1,433)	702, 731	1,433	239	607	125	1,307	533	138
Model 1 (excluding body iron)								
β Coefficient	–0.134	–0.020	0.543	0.790	0.849	0.175	0.206	
Adjusted OR (95% CI)	0.875	0.980	1.722	2.203	2.338	1.192	1.229	
	(0.64–1.19)	(0.91–1.05)	(1.19–2.48)	(1.61–2.99)	(1.50–3.66)	(0.68–2.08)	(0.89–1.69)	
P value	0.393	0.569	**0.004**	**< 0.001**	**< 0.001**	0.539	0.200	
Model 2 (including body iron)								
β Coefficient	–0.144	–0.021	0.341	0.782	0.786	0.156	0.268	0.973
Adjusted OR (95% CI)	0.866	0.979	1.406	2.186	2.195	1.169	1.308	2.646
	(0.64–1.18)	(0.91–1.05)	(0.96–2.06)	(1.60–2.98)	(1.40–3.45)	(0.86–1.59)	(0.95–1.80)	(1.72–4.06)
P value	0.361	0.565	0.081	**< 0.001**	**< 0.001**	0.324	0.101	**< 0.001**

AGP = α-1-acid-glycoprotein; CI = confidence interval; CRP = C-reactive protein; OR = odds ratio; RBP = retinol-binding protein; VA = vitamin A. Bold values = statistically significant.
*Defined by HbE > 5%.
†0.7 ≤ RBP1 < 1.05 μmol/L.
‡Serum zinc < 9.9 μmol/L for age 4–9 years, < 10.1 μmol/L for girls ≥ 10 years of age, < 10.7 μmol/L for boys ≥ 10 years of age.
§Elevated CRP/AGP or both CRP and AGP.
¶Body iron < 4 mg/kg body weight.

24.8% to 62.2%, and the prevalence of hookworm ranged from 15.3% to 68.9%.[22] This suggests that the deworming program, with twice annual mebendazole treatments, has effectively reduced *Ascaris* and other STH infection rates but not hookworm infection rates. A recent study from Lao People's Democratic Republic indicated mebendazole to have low effectiveness against hookworm infection,[46] supporting the findings of the present study.

The important finding that hookworm infection contributed to lower scores in all three cognitive performance tests executed requires further exploration. Several pathways by which intestinal parasite infection leads to lower cognitive test scores are conceivable; for example, chronic blood loss resulting from parasitic infection can lead to lower micronutrient status and in turn, lower cognitive test scores. Another possibility is that chronic inflammation and illness lead to decreased iron absorption and increased absenteeism. In the present study, we show that hookworm infection led to lower iron status, and children with parasite infection had a much higher risk for negative or marginal body iron. Indeed, the intensity of hookworm infection was directly related to body iron. Hookworm infection, however, was not associated with markers of systemic inflammation (the acute phase proteins CRP and AGP) suggesting that acute or chronic inflammation did not play a role in the lower cognitive test scores in the children with hookworm infection. We cannot exclude the possibility that local intestinal inflammation caused by the hookworm infection has affected iron absorption, but chronic blood loss by hookworm infection leading to lower micronutrient status appears the most likely pathway in our study. Few studies have reported the association between hookworm infection and inflammation. However, one study with Ivorian school-aged children supports our findings, as it showed that hookworm infection did not produce systemic inflammation or increase serum hepcidin concentrations, nor did it influence iron absorption and utilization.[47]

In the present study, hookworm infection affected iron status, but not zinc or VA status. Other studies found reduced serum retinol concentrations in helminth-infected children, and such deficits were attributed to *Ascaris* infection.[48,49] When *Ascaris*-induced intestinal obstruction extends to the bile duct, the malabsorption of fat-soluble vitamins such as VA can be impaired. In one Kenyan study, an association was found between *Ascaris* and *Trichuris* infection and VAD among preschool children residing in an urban slum.[50] Another study reported *Trichuris* infection to be a negative predictor of serum zinc concentration.[51] However, in the present study, intestinal parasite infection was dominated by hookworm (95%) and the contribution of other parasites such as *Ascaris* and *Trichuris* was very low (< 1%); this difference may therefore explain the lack of association between hookworm infection and VA or zinc status within our cohort.

Hookworm infection was also a significant risk factor for anemia. Similar to cognition, hookworm infection could impact anemia prevalence through reductions in body iron or through "anemia of chronic infection" where the inflammatory response leads to a redistribution of iron to the macrophages, finally leading to anemia. In our analyses, hookworm infection was only a significant risk factor for anemia when body iron was not included. Inflammation had no impact on anemia prevalence. These results suggest that

hookworm infection did not have an effect on anemia through inflammation; instead its contribution was through the direct effect on body iron. Studies in east African preschool children clearly demonstrated a strong correlation between hookworm infection and anemia. In a community in coastal Kenya, severe anemia was associated with hookworm infections (>200 epg) at all ages (range = 6–76 months) and in both genders.[52] In Zanzibar, there was also an association between the intensity of hookworm infection and other ID indicators, such as serum FER and erythrocyte protoporphyrin.[53] Theoretical calculations on the quantity of blood lost through hookworm infections concluded that hookworm could be a leading cause of ID in schoolchildren.[54] Another study in Kenyan children and adults indicated that even light hookworm infections contributed significantly to lower Hb and serum FER concentrations in the studied adult population.[55] Some studies suggest, however, that the effect of hookworm on Hb concentrations is not entirely mediated by low iron stores, but that nutritional deficiencies of folic acid, vitamin B12, and VA are also implicated. However, we did not find proof of this in our cohort of Cambodia schoolchildren.

To conclude, STH infection within Cambodian schoolchildren, primarily hookworm, was associated with lower cognitive test scores and lower body iron but not lower VA or zinc status. Hookworm infection was a risk factor for anemia, but this effect was mainly mediated through the effect on body iron. Interventions that are more effective against hookworm infection are needed for Cambodian schoolchildren to contribute to better health and improvement of overall cognitive performance.

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